THE MENTALITY OF THE AUSTRALIAN ABORIGINE

by

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The Australian aborigine, in common with the examples of his native flora and fauna, represents a biological species which existed for a long period apart from world competition. He may be expected, therefore, to exhibit primitive features representing a survival of anatomical and mental characteristics of a former era in the evolution of the more culturally developed human races of the present day.

Such anatomical features do exist, and the most important of them are found in the skull and brain. The differences in the teeth, facial bones, and contour of the superciliary ridges do not concern our present enquiry, but the small capacity of the cranial cavity is a pertinent fact. This capacity has been measured by a number of observers,[1] and their figures show an average of about 1,290 c.c. for the male and 1,140 c.c. for the female. The extreme ranges in published measurements are 1,590 c.c. (Krause) and 930 c.c. (Turner). Compared with a European average of 1,500 c.c. males, 1,340 c.c. females, the averages of the Australian native are low, and only equalled by those of the African Bushman and the Andaman Islanders. As all authorities recognize a direct correlation of cranial capacity with height (the chief factor in sex difference in capacity), and as these two latter races are of small stature, and the Australian of medium height, it follows that the cranial capacity of the aborigine is relatively less than that of any other extant race.

The primitive features of the brain itself are exhibited in several characters, of which the most important are the small size, the exposure of the insula in many cases, and the presence of a distinct *sulcus lunatus*, with operculation of the visual cortex, in the majority of examples (6) (8) (24). The last feature is present to

[1] The following averages were found, the figures in square brackets indicating the number of observations: **Males:** Broca (1) 1347, Quatrefages (17) [22] 1330, Turner (22) [20] 1293, Sarasin (18) 1310. **Females:** Broca 1181, Quatrefages [16] 1185, Turner [10] 1103, Krause (13) [5] 1136, Sarasin 1154. **Mixed:** Davis (5) [22] 1320, Turner [30] 1230, Quatrefages [38] 1240, Flower (9) [16] 1298, Krause [60] 1238, Duckworth (7) [150] 1246, Wood Jones (12) [90] 1290. (The last calculated by Lee Formula. The others measured by means of shot, sand, seed, or water.) As the sex characters are not definite, the mixed results are subject to least error.

a less extent in the brains of some other primitive races (Elliot Smith). It is also indicated in the cast of the cranial cavity of the Neanderthal skull.

With regard to the actual weight of the aboriginal brain, three male cases recorded by Woollard (24), showing an average of 1,101·9 grms. with a range from 1,138 to 1,044·6 grms., are apparently the only measured weights published. The commonly quoted figure of 1,185 grms. (16) (23) (6) is an estimated weight calculated by Barnard Davis from cranial capacity. The European average is about 1,330 grms., the male brain averaging 1,400 grms., the female 1,260 grms.

Most authorities deny any correlation between cranial capacity and mental ability, but these investigators have dealt with data concerning individuals of equal cultural or racial status. There is undoubtedly a positive correlation between intelligence and cranial capacity, and consequent brain weight, in the evolutionary history of types of animal life. Berry and Porteus (2) have shown that the same correlation is strongly marked in the case of mental defectives. Todd (21) found a low cranial capacity in his material "recruited from the waterfront, the criminal districts, and the underworld." Porteus (14) found a correlation of +0·3 in his investigations in Honolulu. Elliot Smith (19) has stated, "it is doubtful if normal intellectual functions could be carried on in a brain which weighs less than 960 grammes." The element of brain weight must not be overestimated, however, as the brain of Anatole France weighed only 1,017 grms. (11), and on the other hand the average brain weight of the Esquimaux and the Pelew Islanders is greater than the European (6).

Any attempt to gauge the intelligence of the aborigine is beset with great difficulties. In the first place, "intelligence" is a word which has a common-sense meaning, but which is incapable of exact definition. In recent years it has become associated with a large field of psychological research. The growing intelligence of the normal child makes it possible for the child, as age advances, (1) to master tasks demanding greater power of analysis and deduction, and of memory, (2) to master tasks demanding a wider field of knowledge, (3) to master tasks of equal difficulty more quickly. In this way it is possible to grade intelligence in accordance with the mental capacity of a normal child of a certain number of years of age, and, by using this factor as a unit of measurement, to exploit "intelligence" mathematically.

As a child grows in age it also grows in stature and weight, in muscular strength, and in efficiency in co-ordinated movements and sensory discrimination. Determinations of these factors in children, therefore, show a correlation with intelligence, as indeed does any extreme instance of concomitant variation, such as the size of a child's boots. Naturally these determinations cannot be expected to correlate with intelligence when adults are compared in "mental age" one with another. Also actual intelligence tests of both children and adults cannot be correlated with the unit measurement expressed in mental age unless such tests are applied to individuals possessing the same amount of

mental preparation. Thus Thorndike (20) states: "Intellectual tasks, success, in which requires zero training, and is uninfluenced by any kind or amount of training, do not exist, and cannot exist." "The problem of analyzing a person's intellectual ability into an amount due to nature and an amount due to nurture is unsolved. No task or test has been proved to be a measure of the former alone."

This is the dilemma which appears when the mental ability of the Australian native is subjected to observation in field work.

The aborigine is totally unacquainted with geometrical, mathematical, grammatical, pictorial, or mechanical problems, such as form the usual basis of systems for testing intelligence. Also it is hard to interest him sufficiently to make any real attempt at any such problems. The Porteus mazes have proved to be the most interesting of the normal tests, and therefore the one which presents the best prospects of success, but the "normally prepared" mind is not being tested, and the task is abnormally hard for the native. While watching a native at work on these problems one can almost feel the intensity of the mental strain involved.

Burt's (3) Graded Reasoning Tests would appear to be a possible clue to a fair system of mental testing of individuals of different race and civilization, but such a system has yet to be devised.

Children of full-blooded aboriginal parents and of mixed black and caste parentage are educated at various mission stations on terms of equality. Porteus (15) made an investigation of such children at Point MacLeay, in South Australia, with the maze tests. He states that few of the children tested were full-blooded. Twenty-eight children were examined, and he found "that the younger children succeeded, relatively, far better than the older ones. Of 12 children who were over 11 years of age 9 were retarded by the tests, the total deficiency being 16 years. Of the 16 children under 11 years of age, 6 passed tests above their chronological age, 9 passed 'at age,' and 1 was retarded one year. . . . This also accords with the experience of the teacher of the school, who says that he finds little difficulty in bringing his pupils through the lower grades, but finds it very difficult to educate them beyond the fourth grade, or midway through the school course."

At Hermansberg, in Central Australia, one of us (10) gave a scholastic examination to 20 full-blooded and 10 three-quarter caste children. The schooling could not be compared for many reasons with that of ordinary schools, so the results of the full-blooded and caste children were compared in groups of sexes of equivalent age and length of time at school. It was found that "The most definite result of this grouping was to show a superiority of scholastic attainment by the senior boys with white blood over that of their full-blooded companions. It is tempting to draw a conclusion that the full-blooded children are retarded in mental growth with increasing age to a greater degree than

children with some degree of European ancestry. The numbers are too few to warrant this deduction, however."

This represents the limit of our ability to gauge at present the ability of the aborigine in terms of the European standards, unless, reverting to cranial capacities, the finding of Berry and Porteus (2) that "the Australian aborigine has an average brain capacity between the normal medians for twelve and thirteen years," be stretched beyond its intended meaning. These authors do not state that this is the average mental ability, for in the first place brain capacity and intelligence are not correlated as exactly as this, and secondly there is a possibility that the Lee formula number 14, on which the capacity was calculated, is not quite applicable to the Australian native (cf. Todd (21), who found the Lee formula inaccurate in the case of the negro skull).

The main subject matter of the present paper is concerned with the results which have been obtained in "psychology" during a visit of the Adelaide University Anthropological Expedition to Central Australia in August, 1930. The expedition is organized on the team plan, and the visits only extend over two or three weeks. Therefore, any investigations in the region of psychology need to be as direct as possible. Under these circumstances the simpler experiments of experimental psychology have been the primary objective, as they at least give some indication of the perceptual basis on which the mental life is built up, and of the motor co-ordinations in which the mind gains expression. The reaction of the natives to some more directly intellectual tasks has been investigated also, in the hope that they may reveal some data which may be of interest. Pertinent facts are also gleaned from the general work of the party.

The expedition camped at MacDonald Downs, about 200 miles N.E. of Alice Springs. The test subjects were full-blooded natives of the Iliaura tribe. Most had made their first acquaintance with white people in the last six years, and could speak no English at all; none had command of more than a few words of English. The two sons and two daughters of the station owner (Mr. Chalmers) spoke the native language fluently, and acted as interpreters for us. Our thanks are due to them for their very valuable assistance.

A. MOTOR TESTS.

Tests 1 to 6 below were carried out by the methods described by Whipple, "Manual of Mental and Physical Tests" (3rd ed., 1924), pp. 100-130.

1. *Strength of Grip.*

Adult males (average of 12 subjects: Right hand, 41·0 kilos.; left hand, 37·7 kilos.

Adult females (average of 8 subjects): Right hand, 26·1 kilos; left hand, 26·0 kilos.

These male readings are 16·5% less than Whipple's norms for boys of 18 years. The females show 12% less for the right hand, and 6·5% less for the left hand, in comparison with the norms for girls of 18 years.

2. *Strength of Back.*

Adult males (average of 12 subjects) : 92·5 kg. Norm, 150 kg.
Adult females (average of 5 subjects) : 61·0 kg.

3. *Strength of Legs.*

Adult males (average of 12 subjects) : 109·5 kg. Norm, 189·5 kg.
Adult females (average of 5 subjects) : 61·0 kg.

4. *Ergographic Readings.*

Sixteen grips at intervals of 4 seconds. Recorded readings were divided in each case into four successive groups of four, and the average of each group calculated. The following are the averages of such groups (in kilogrammes) :
Adult males (9 subjects) : 32, 28·8, 27·5, 26·7.
Adult females (5 subjects) : 18·3, 17·1, 18·0, 18·3.

5. *Endurance Test.*

Gripping dynamometer for 60 seconds. Readings at 4-second intervals, grouped and averaged as above.
Adult males (10 cases) : 27·8, 21·0, 19·4, 17·6.
Adult females (7 cases : 20·9, 15·1, 12·7, 12·1.

One outstanding exception in the strength tests was Number 4, a male about 27 years of age. His grips were 76·5 right and 59 left. Strength of back 200, of legs 182. The next best results were : Grip 54·5, back 127, legs 145.

The natives are not of a muscular type as a rule, and pulling is almost unrepresented in their lives. Endurance is, however, a strong feature. The women will walk many miles in a day, carrying a heavy child and numerous impedimenta.

6. *Speed of Movement. Tapping Tests.*

The following are the average results obtained, representing the number of taps in successive intervals of 10 seconds, and the total for 30 seconds :

Males (13 cases) : Right hand, 58·2, 51·5, 50·1 ; total, 159·8. Left hand, 52·2, 46·9, 44·5 ; total, 143·6.

Females (13 cases) : Right hand, 54·3, 50·8, 47·2 ; total, 152·3. Left hand, 51·1, 44·8, 45·0 ; total, 140·9.

The slowest record obtained was that of Number 10, male, 48 years, who tapped 38, 36, 36 right, and 36, 36, 38 left. This was an instance where dignity outweighed the spirit of emulation. The best male score was 204, the best female 173.

Three male children's ages and scores were: 6 years, 137 R., 132 L.; 10 years, 165 R., 129 L.; 12 years, 184 R., 141 L. Three female children scored: 11 years, 129 R., 114 L.; 12 years, 149 R., 120 L.; 14 years, 151 R., and 139 L. These male figures are in fair agreement with Whipple's norms, but the female results are about 12% belows his norms for children.

7. *Speed and Accuracy of Movements. Peg Board.*

The peg board was an exact copy of that described by Hull ("Aptitude Testing" (undated), pp. 74, 75). Twenty-four matches have to be taken one by one from a magazine and inserted vertically in twenty-four holes in the board. This test has to be performed as quickly as possible. The best time recorded by members of the University party was 45 seconds; other times ranged up to 62 seconds.

The following figures represent the average times taken in seconds at the first, second, and third attempts. The figures in brackets indicate the number of subjects represented in the averages. The experiments were sometimes disturbed, so the latter figures are variable:

Males: Right hand, (7) 76·4, (8) 64·4, (5) 63·0. Left hand, (8) 72·2, (8) 68·5, (4) 68·8.

Females: Right hand, (6) 78·3, (6) 69·0. Left hand, (7) 80·6, (6) 70·0.

The similarity of right and left hand results is notable. One male (Number 1) did the test in 45 seconds with his right hand, although left-handed. (This man could evert the upper lid of either eye with the thumb and forefinger of either hand.) Three other males and two females accomplished it in 60 seconds or under.

These tests are not really suitable to the aborigine. The skill and quickness shown by the men in dodging weapons and spinning discs of bark, and by the women in digging and winnowing, leave no doubt that the aborigine's motor co-ordination is of a high order, and beyond that brought out in our standardized tests.

A very pretty exhibition of manual dexterity was given by the boys in a game they used to play round a blazing fire at night. Gum leaves were bent and trimmed into a resemblance to a propeller. These were then thrown, spinning horizontally, into the hot air current over the fire, and flew upwards on this, falling still spinning, and possibly spinning up again in another current. This is a dexterity test that very few Europeans could accomplish. A further instance may be mentioned. In making a surprise attack on a camp, a native will advance, holding a spear in his toes and trailing it unseen in the grass, until at the moment of attack it is whipped up into the hand and thrown.

Attempts to determine reaction time on this visit were quite unsuccessful, no determination coming within approximate distance of their natural reaction time as indicated above.

B. SENSORY TESTS.

1. *Visual Acuity.*

This is of particular interest, in view of the operculation of the aborigine's visual cortex and of the skill which he shows in tracking. A mounted tracker follows a barefoot trail over stony ground without dismounting and without apparent close attention to the tracks. This accomplishment is analogous to the reading of the educated person, who skims the pages of a book, following the meaning, but not noticing each individual letter of the text. The eyesight of natives past middle age usually is dimmed by traumata or conjunctivitis. At Hermansberg evidence was obtained of acuity of vision of native children more than twice that of the best, and three times that of the average, white tested. These children were keenly competitive during the observations. On the present occasion this keenness was not exhibited, and no results better than those of the whites were obtained.

2. *Colour Blindness.*

No case of colour blindness has been found on this or any previous expedition.

3. *Colour Nomenclature.*

Many primitive people have no name for blue. This was apparently the case at Hermansberg, with the Aranda, but a word meaning "like fire smoke" was coined for our benefit. The Iliaura on the present occasion used many terms likening shades of colours to different objects or phenomena, e.g., hot ashes, various ochres, various flowers, the sky, the sunset; a royal blue was called "ingua 'binya," like the dark night. Actual colour names appear to be used only for black, white, red, yellow, and green. Blue was called variously "enbar," i.e., no name; "irabulla," i.e., black; or "alkara abinya," i.e., like the sky; and "ingua 'binya."

4. *Spatial Perception.*

Various tests were made to measure the degree to which the natives are subject to the illusion of shortening which is experienced when horizontal and vertical lines are compared in length, and when arrow-head lines are added to horizontal lines. A large series of results (10) was obtained at Hermansberg, and the native response found to be equal to that of Europeans. Difficulty was experienced in making the Iliaura people understand the test; three or four results only were obtained, and these were within the range of European results.

On the other hand, these natives, like the Hermansberg natives without schooling, failed to see an illusion in pictures drawn on a background in strong perspective.

5. *Acuity of Hearing.*

This was not tested with apparatus, but on several occasions the noise of a distant approaching motor car was picked up by natives and whites simultaneously.

The upper range of audition was tested with a Galton whistle. The natives' range was equal to, and did not exceed, that of the younger members of the University party.

6. *Tactile Sensation.*

This was tested by means of a battery of von Frey's hairs. These exerted a pressure in grames per square millimetre as follows: 3·7, 4·25, 4·85, 5·5, 7·4, 8·5, 12·7, 14·65, 17·75, 25·5, 30·6, and 33·0 respectively. These figures are subject to experimental error, as the hairs were measured in two diameters with a Starrett micrometer reading to 0·0005 inch only, and the finer hairs ranged from 0·0030 to 0·0055 inch in diameter. Also the hairs are not quite straight, and the bending factor, when pressed on a mobile scale pan, is not constant; the mean of a number of readings was taken. The perpendicularity of cross section was tested by shadow projection.

The median results for nine adult males and seven adult females were as follows:

	Lip.	Nape of Neck.	Tip of R. Index Finger.	Dorsum of Hand.	Mid. Ant. Surface Forearm.	Mid. Ext. Surface Lower Leg.	Mid. Sole of Foot.
Males ..	3·7	7·4	12·7	7·4	12·7	12·7	25·5
Females ..	3·7	3·7	7·4	4·25	7·4	7·4	17·75

Myers ("Text-book of Experimental Psychology" (1925), pt. 1, p. 240) gives the following figures: Lip, 2·5; shoulder, 16; finger tip, 3; dorsal surface of finger, 5; forearm, 8; shin and sole, 28.

The only very marked divergence in the native results is in the case of the finger tip, but a manual worker gives results conformable to that of the native in this instance. The sensitiveness of the sole and lower leg, which are unprotected, is remarkable.

At Hermansberg, where many of the natives wear boots, a higher reading for the sole was obtained.

7. *Aesthesiometry.*

A light pair of compass points was used, and the separation of the points measured on a millimeter scale. The constant method was employed, ten or more tests being made at each of various separation intervals. Frequent control applications of one point only were interspersed. The points were applied longitudinally in the middle of the flexor surface of the right forearm. After a preliminary explanatory test, the subject's eyes were screened by an assistant for the experimental test.

Myers sets the limen at the average point of 50%, Whipple at the point of 80%, of correct answers. The results obtained were: Males (8 subjects): Myers Limen—Average, 53·4; maximum, 75; minimum, 25. Whipple Limen—Average, 64·4; maximum, 85; minimum, 30. Females (4 subjects): Myers Limen—Average, 34·8; maximum, 38; minimum, 32·25. Whipple Limen—Average, 45; maximum, 50; minimum, 40.

Myers (ibid., p. 218) gives an average limen of 40, Whipple (ibid., p. 251) an average limen of 40·5.

The control figures in this test are interesting. When touched with one point the figures for "correct answers/incorrect answers" were, for the males, 23/5, 16/3, 22/6, 22/0, 17/0, 12/2, 16/0, and 15/2 respectively, and for the females 15/9, 18/13, 12/3, 13/2, but in addition results from four other females were discarded, as in three cases the above ratios were 2/4, 6/8, and 15/15, while the fourth could not be induced to signal two points even at a separation of 150 mm. In two of the four female results admitted, the limen was less defined than in the case of the males and other two females, corresponding to the control indications.

8. *Discrimination of Weights.*

Twenty-four similar wooden pill boxes were packed with cotton wool and lead to gross weights varying from 80 to 120 grammes (*vide* Whipple, ibid., p. 224). The boxes were marked in a six-figure cypher, and the constant method used without screening the eyes. The results were very poor. Two males showed a 50% limen of about 1 in 32, one male 1 in 16, two females about 1 in 9 to 1 in 7. One other male and female quickly lapsed into selecting the left-hand box every time. The normal white limen is about 1 in 40 to 1 in 16, with extremes of 1 in 80 to 1 in 5.

9. *Sensation of Pain.*

It is usually considered that the aborigines are insensitive to pain, as they receive severe injuries without great complaint. Pain sensation was measured by applying the nail of the right index finger against a flat brass stop 5 mm. x 6 mm. in surface dimension, and then exerting pressure on the opposing flexor surface of the finger by means of the handle of the dynamometer, which was screwed up by a suspending threaded staple and butterfly nut. The pressure at which the subject made complaint was read off on the dynamometer scale.

The following figures were obtained:

	Average.	Maximum.	Minimum.
Natives:			
Adult Males (12 subjects) ..	6·72 kg.	10·0 kg.	3·2 kg.
Adult Females (7 subjects) ..	5·78 „	6·8 „	4·5 „
Whites:			
Central Australians (6) ..	5·73 „	6·6 „	4·5 „
University Party (8)	4·82 „	5·9 „	3·9 „

The native is therefore definitely less sensitive to pain than the whites on an average, but the lower threshholds are much the same, and the insensitivity of the aborigine is not as marked as is commonly assumed.

In regard to the relative sensitivity of the native to psychic and traumatic pain, a comment of some natives that they disliked the chattering willy wagtails because they were always scolding, was followed by the remark that the women much preferred to be beaten than growled at. The informants were, however, males.

C. MENTAL TESTS.

Even the simplest sensory and motor tests involve a certain degree of mental application and ability, and it is noticeable that the women react poorly as a whole in comparison with the men. The controls in the aesthesiometry tests show this very plainly. At Hermansberg the same was evident, some of the women there, in a test for colour preference, could not be induced even to exhibit sufficient mental activity to select beads of one colour rather than another but just appropriated the nearest sample. This dullness on the part of the women would appear to be due to the absence of intellectual stimulation in their lives. The women carry out the drudgery of collecting food and rearing children. The wider interests of the men in hunting, fighting, councils, and ceremonies, which involve much preparation and discussion in addition to the actual performance, keep the men more alert mentally.

Some tests were made to find the natives' reaction to more or less standardized intellectual tasks, chiefly of the "performance" type.

1. *Puzzles.*

Four of these were used:

(i) Goddard's form board. (*Vide* Whipple, ibid., p. 298.) This is a test for children of four years, and when done against time is performed by the average European child in 30 seconds at five years of age, 16 seconds at twelve years of age.

(ii) A puzzle similar to the above (illustrated in Transactions of the Royal Society of South Australia, liv (1930), p. 94), and found in practice to be slightly more difficult than the Goddard.

(iii) Healy Construction Test A (Healy, "The Individual Delinquent" (1915), p. 88). This usually is credited as a ten-year-old test.

(iv) Healy Construction Test B (Healy, ibid., p. 89), which is given a status of a twelve-year-old test.

The results are appended as Table 1. The subjects are classified in order of speed merit in the four-year-old tests. It will be seen that this has no correlation with the success in the Healy tests. The attempts of the natives obviously

cannot be interpreted in terms of the normal achievements of European children.

2. *Porteus Mazes.*

Tests with the mazes "caught on" more successfully than any other performance test. The maze for five years was used to explain the nature of the problem, stated in terms of a cattle yard and an animal trying to escape. The subject was given a pencil and a copy of the six-year maze for a trial, and he was sometimes helped in this. No more help was given after this, except to indicate the starting point and the final point of exit. He was provided with a fresh copy of the six-year maze, and then with successive mazes until the limit of success was attained. If the subject made a bad mistake a new copy of the same maze was provided and a fresh start made. In the strict method of marking, any transgression in the wrong direction, beyond the exact line of a port of exit or entry, is counted a mistake in scoring. The native is quite unaccustomed to the use of a pencil. Allowances were made therefore; if the transgression amounted to 1 or 2 millimetres only a "slip" was recorded, and this was not counted in scoring. If the pencil was carried on farther, but the error noticed quickly before it became obvious, or before any trap was entered, an "error" was recorded with half penalty. A larger mistake was recorded as a "failure," with full penalty.

Table 2 (appended) gives the results obtained. The small letters, a, b, c, d, represent successive attempts at the same maze, R indicates a complete success, E an error, S a slip, and F a failure, as defined above. The final column gives the score according to the Porteus method, modified as stated. The score can be marked, however, as desired, as the record gives full particulars of the results.

The score would be read as "mental ages" in the case of European children.

3. *Blot and Footprint Test.*

A large part of the aborigine's natural education is in reading tracks. A test was devised along these lines, in the hope of bringing out his own particular abilities. Thirty footprints were prepared in Adelaide by the technique of taking dermatoglyphs of the hand. Thirty indifferent blots were prepared on writing paper squares by dropping a piece of cotton wool, wetted with ink, six times on each square. The subject was shown ten specimens of blots, or dermatoglyphs, for 10 seconds each. These ten were mixed with the twenty remaining copies, and then the copies were dealt out one by one in front of the native, and he was asked to pick the ones he had seen before. The numbers of correctly and incorrectly selected designs were noted. Twenty-two trials were made, but the test was a failure. It was too pictorial. The result was entirely negative; a total of 101 designs were correctly chosen and 150 incorrectly chosen.

D. EXPRESSION OF EMOTION.

The conventional test with a recording grip dynamometer was used. The subject made a maximal grip for 60 seconds with the eyes closed. The experiment was repeated, but first attar of roses, then strong smelling-salts were applied to the nostrils (cf. Myers, ibid., pt. ii, p. 117).

The second tracing was unaffected, even though the ammoniacal fumes caused lachrimation.

Mr. N. B. Tindale, who was collecting phonograph records of native songs, found two of a new type during this expedition. Both contained an emotional content in both words and music. Both were sung by Number 3. The first, which was sung with quite a lilt, related how happy everyone was in the green time. The second mourned how heavy were the water-bags on the long march in the heat, and how everyone cried.

E. NATURAL EXPRESSIONS OF INGENUITY.

The following instances of native ingenuity may be mentioned.

It was found at Hermansberg (4) that the aborigine diagnoses real from feigned death of an enemy by a method which is based on a sound physiological principle. He tests for relaxation of the anal sphincter with a spear. For another instance I am indebted to Mr. A. McLean, of Stuart's Creek. In adapting a tin pail as a water vessel on a journey, the native packs some grass into the pail to prevent the water from swilling over.

The natives have developed a complex social organization. Elaborate geometrical diagrams are necessary to give expression to the various forms of this. Moreover, the natives readily solve problems arising from inter-marriage of tribes having different forms of organization (10).

F. REASONABLENESS.

The native does not always act on impulse, like a crowd at a football match may do. In Bathurst Island, in 1913, one of us was suspected of appropriating some ornament of an initiate. This misunderstanding arose from our mutual limitations of language. The offence was a serious one, and it was suggested that the supposed culprit should "fall into a crocodile pool." Instead of taking precipitate action in response to the emotional antagonism aroused, the men sat down in council and talked the matter over. As a result of their deliberations the accused was acquitted and full friendly relations re-established immediately.

SUMMARY.

The present paper gives some more definite data concerning the motor and sensory abilities and attributes of the Australian aborigine, and at this level he or she does not differ much from the European.

The difficulty of estimating mental abilities is discussed, and some data brought together which have some bearing on the question.

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APPENDIX.

TABLE 1.

Puzzles.

Serial No. & Sex.		"Goddard."		"Landscape."	"Healy A."	"Healy B."
4	M.	Direct	0m36	0m35	2m10	All but top square in 4m.
42	F.	Direct	0m52	1m40	Failed	
6	M.	Direct	0m52	7/9 1m20	Failed	
31	F.	7 tried in 5 9 tried in 3	1m3	1m2	Failed	
1	M.	Direct Time missed.		1m20	0m25	1m15
5	M.	Direct Time missed.		0m55	Failed	
36	F.	Direct	1m8	1m22	Failed	
41	F.	Direct	1m7	3/9 3m		
32	F.	6 tried in 7	1m23	1m28	0m45	Half top line and small rectangle unsolved, 2m30
38	M.	2 wrong way many times. 6 tried in 7	1m50	1m30	0m30	One piece top line and two small rectangles unsolved, 3m.

TABLE 2.

Porteus Mazes.

Serial No.	Sex and Age.	VI	VII	VIII	IX	X	XI	XII	XIV	Score in Points
1	M. 30	a.R	a.R	a.R	a.R	a.R	a.R	a.R	a.R	16
42	F. 24	a.R	a.R	a.R	a.R	a.F b.1S	a.F b.R	a.F b.1S	a,b.F c.R	14
3	M. 20	a.R	a.R	a.R	a.R	a.R	a.1S	a,b.F c.1E	a,b,c.F d.2S	13·25
4	M. 27	a.R	a.R	a.R	a.R	a.F b.R	a.F b.R	a.R	a,b,c,d.F	13
38	M. 15	a.R	a.R	a.R	a.1E b.R	a.R	a.R	a.F b.R	a,b,c,d.F	13·25
15	M. 30	a.R	a.R	a.R	a.R	a.F b.1S	a.1S	a,b,c,d.F		10·5
5	M. 24	a.R	a.R	a.4S b.R	a.R	a.R	a.F b.1S c.R	a,b,c,d.F		10·5
32	F. 17	a.R	a.R	a.1E	a.R	a.F b.R	a.F b.1E	a,b,c,d.F		9·5
14	M. 35	a.R	a.R	a.R	a.R	a.R	a,b,c,d.F			10
18	M. 30	a.R	a.R	a.1E	a.R	a.R	a,b,c.F			9·75

Serial No.	Sex and Age.	VI	VII	VIII	IX	X	XI	XII	XIV	Score in Points
36	F. 14	a.R	a.R	a.F b.R	a.R	a.F b.R	a,b,c,d.F			9
41	F. 18	a.R	a.R	a.F b.R	a.R	a.F b.R	a,b,c,d.F			9
13	M. 25	a.R	a.R	a.F b.R	a.R	a,b,c.F				8·5
40	F. 18	a.R	a.R	a,b,c.F						7
37	F. 18	a.F b.18	a.R	a,b,c.F						6·5
20	M. 30	a.R	a.F b.R	a,b,c.F						6·5
6	M. 30	a,b,c.F								
31	F. 35	a,b.F								

THE REGULATION OF THE SALT CONTENTS OF THE BLOOD OF AQUATIC ANIMALS, AND THE PROBLEM OF THE PERMEABILITY OF THE BOUNDING MEMBRANES OF AQUATIC INVERTEBRATES

by

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INTRODUCTION.

It is gradually becoming appreciated by zoologists that the salinity of the body fluids of marine invertebrates is very like that of the seawater in which they are living. The osmotic pressure of the blood is nearly identical with that of the external medium. When such marine creatures (e.g., polychaete worms, crustacea, molluscs, echinoderms) are placed in diluted seawater there is at once a corresponding drop in the salinity of the blood or coelomic fluid. Conversely, if the salinity of seawater is increased by the addition of appropriate salts, the blood salinity of such animals submerged in this medium becomes higher. A few years ago the evidence seemed all to point to the fact that this decrease or increase took place entirely owing to the passage of water through the bounding membranes of the body-walls, gills, etc. Such bounding membranes were judged to be quite impermeable to salts by Frédéricq (1901, 1904), Bottazzi (1908), and others.

A starfish taken from seawater of normal ocean salinity ($35^0/_{00}$) and placed in a mixture of this water with distilled water (say, two-thirds sea to one-third fresh water) will increase in weight, and may even be seen to swell. The salinity of its coelomic fluid diminishes. The increased weight clearly represents the water which has diffused through the bounding membranes of the starfish, so there is no question about the permeability of these membranes to water. But the change in salinity of the coelomic fluid could be produced in three ways: (1) by the diffusion of water from without under osmotic potentials; (2) by the passage outwards of some of the salt or ions; and (3) by the combination of both processes. For various reasons it is not easy to be certain in cases (2) and (3). If, however, the bounding membranes of the marine invertebrates

were equally permeable to water and salts (or the ions of the latter) one would not find the increase in weight in diluted water and the decrease in concentrated water, which is a very general occurrence. Evidently in most cases the permeability to salts, if actual, is of a much lower order than the permeability to water. Thus Dekhuyzen (1920) found that *Phascolosoma vulgare* of 3·125 grammes weight increased to 3·47 grammes in distilled water in 5 minutes, and that the amount of body fluid had then increased by 18·8%, and at the end of the experiment the surrounding distilled water contained only the slightest trace of chlorine. Many other experiments made on similar lines (Henri et Lalou 1904, Bottazzi and Enriques 1901) have been regarded as supporting the theory of the *semi*-permeability of the bounding membranes of aquatic invertebrates.

Up to date very few species of aquatic invertebrates have been thoroughly investigated, and only two are marine species. The fact that we are working in Australia has compelled us to utilize different genera altogether from those previously studied. Again, it is well known that some marine invertebrates can withstand great variations in the salinity of the seawater. These often occur dominantly in brackish water, and are manifestly more suitable for experiments than sensitive restricted species. For this reason *Carcinus moenas*, the common European short crab, has been one of the chief experimental types in the past. We have the opportunity of using certain marine invertebrates of estuarine mangrove swamps which are exposed to considerable changes in salinity after heavy rains. Hence our work increases the range of material studied. It has included three mangrove crabs, a new freshwater crab not yet named (a new Brachyuran genus in fresh water), the Australian fresh-water crayfishes, and the interesting aquatic slug, *Onchidium*, which appeared on one mangrove swamp in hundreds of thousands.

TECHNIQUE.

The osmotic pressure has been determined by the freezing point apparatus of Beckmann. Chemical analyses of blood have been made by methods noted in the text. The essential feature of our work has been to keep the experimental animals in as healthy a condition as possible. To this end we set up a temporary laboratory during the summer actually on the edge of a mangrove swamp. The cases noted below are only a few out of many experiments, for owing to technical difficulties this investigation has extended for over two years, and setbacks often occurred. Any doubtful experiment was always repeated several times, and a sharp lookout kept for pathological effects. Seawater dilutions were made with good tap water, except when for special reasons distilled water was used. Some of our most interesting experiments were made in what we call concentrated seawater.

Concentrated seawater was always made up partly of natural seawater and partly of artificial. Two methods were used. It was assumed that a good

approach to natural sea water could be made by dissolving 2,815 grms. of NaCl, 67 grms. of KCl, 551 grms. of $MgCl_2$, and 692 grms. of crystalline $MgSO_4$ in water and then making up to 100 litres. To this would be added 145 grms. of calcium chloride and 25 grms. of sod. bicarbonate made into a thick solution in water.

Now if sea water was used in making the above instead of fresh water, a 200% seawater resulted (i.e., salinity 70‰). Our other method was to double the quantity of the above salts, making a 200% seawater directly, and then adding this stock solution as required to ordinary seawater to make concentrations between 100% and 160%.

The depression of the freezing point below 0°C. is indicated throughout this paper by the customary sign, $\triangle$, thus $\triangle$ of sea water = 1·9 means that the freezing point of the seawater was —1·9°C.

TABLE 1.

Reaction of the Crab, Heloecius cordiformis, to Fresh Water.

Duration of Expt.	Medium.	△ of Medium (°C).	△ of Blood (°C).
2 hours	fresh water	0·0	1·9
6 „	„ „	0·0	1·83
8 „	„ „	0·0	1·7
24 „	„ „	0·0	1·56
30 „	diluted seawater	0·1	1·3
8 weeks	„ „	0·72	1·38
Controls in normal seawater		1·98	1·89

The above is a summary of numerous experiments conducted on *Heloecius cordiformis* as a preliminary to the present paper (the results will be given in detail in another paper by E. Edmonds). They serve to show that this crab resembles *Carcinus moenas* in its reactions to diluted seawater. Both withstand changes of salinity in the external medium with considerable hardiness. Within certain limits the blood is isotonic with the seawater. When, however, the seawater is diluted below these limits the blood salinity becomes more and more different from the external medium the greater the dilution of the latter. Here then is another example of a marine crustacean which is said to be poikilosmotic in the sea (implying that the salinity of the blood increases or diminishes with any changes in the salinity of the water surrounding the animal, and to the same degree, but is homoi-osmotic in diluted seawater.[1]

[1] According to Schlieper (1920) this feature has only been demonstrated thoroughly for *Carcinus moenas* and *Nereis* amongst marine invertebrates which can live, and do so naturally, in brackish water as well as in the sea. The same author believed that this would hold good for all brackish-water forms. We can extend the experimental proof of this, and concur with Schlieper's general belief.

TABLE 2.

Weight of Heloecius cordiformis in Seawater of Salinity 3·5‰.

	Weight at beginning of experiment.	Weight after 1 hour.	Weight after 3 hours.	Weight after 6 hours.	Weight after 72 hours.
Crab E.	8·71 grms.	8·69 grms.	8·68 grms.	8·66 grms.	8·6 grms.
Crab F.	11·21 grms.	11·21 grms.	11·17 grms.	11·20 grms.	11·06 grms.

TABLE 3.

Weight of H. cordiformis in Concentrated Seawater, Salinity 52‰.

	Weight at beginning of experiment.	Weight after 1 hour.	Weight after 3 hours.	Weight after 6 hours.	Weight after 72 hours.
Crab A.	11·61 grms.	11·63 grms.	11·07 grms.	11·07 grms.	—
Crab B.	9·93 grms.	9·88 grms.	9·92 grms.	9·88 grms.	9·64 grms.

These experiments indicate that *Heloecius cordiformis* resembles *Carcinus moenas* in the fact that changes in the composition of the external medium are unattended by changes in weight. The variations noted above occur also in controls, and are due probably to differences in the amount of adherent moisture. To account for the observed changes in the salinity of the blood in similarly conducted experiments a very obvious change in weight would be necessary if the variations in salinity were due to movements of water alone.

These results, taken in conjunction with the freezing point determinations, seem to indicate that internal changes in the blood salinity of this crab are due to the diffusion of salts or their ions. The situation is quite different where "worms," slugs, or echinoderms are concerned, as we shall show later.

TABLE 4.

Response of Holoecius cordiformis to Concentrated Seawater (Salinity, 50‰ and 68‰).

Freezing point of blood in normal seawater. (°C).	△ of concentrated seawater used in experiment. (°C).	Duration of experiment.	△ of Blood. (°C).
—1·9	3·14	38 hours	2·18
	3·14	42 „	2·29
	3·29	36 „	2·26
	4·2	21 „	2·7
	3·38	14 days	2·56
	3·28	28 „	2·92

The series of experiments from which the above examples are taken provides evidence of an extremely interesting difference between our type, *Heloecius cordiformis*, and *Carcinus moenas* and other marine invertebrates. Comparatively few experiments seem to have been made with solutions considerably more concentrated than normal seawater but containing the same ions in the same proportions. Duval kept *Carcinus moenas* in seawater with a △ of 2·8°C., and found that the △ of the blood was approximately the same within 26 hours. From this it has been assumed that homoiosmoticity only occurs in diluted solutions (except

in the case of the brine shrimp, *Artemia*). With *Heloecius*, however, there was never any semblance of the blood's agreeing with the external concentrated seawater after 38 or 42 hours' immersion. Before concluding that our crab was unique in maintaining an independence in blood salinity in high concentrations we endeavoured to keep specimens alive for a longer period. As noted in Table 4, we succeeded. After 14 days the △ of the blood was 2·56°C., the △ of the external medium being 3·28°C., and after 28 days still lower, i.e., 2·92°C. Whether complete isotonicity would eventually be reached is still uncertain, but these experiments are now being continued.

It is clear that there is a greater resistance to the effects of a *diluted* seawater than to a concentrated seawater of a similar difference from the normal. But there is a distinct indication of independence even in the concentrated solutions.

It has been shown that the osmotic pressure of the blood of *Heloecius* increases when crabs are immersed in a more saline seawater. The next point is to discover whether this is due to a passage inwards of salts. For this purpose crabs were placed in four different modifications of seawater. All agreed, however, in possessing approximately the same freezing point, having been made up to this standard. The solutions were: (1) Normal seawater plus concentrated artificial seawater, making about 50‰ salinity; (2) normal seawater plus magnesium sulphate; (3) normal seawater plus urea; (4) normal seawater plus cane sugar.

TABLE 5.

Comparison of Heloecius cordiformis in Magnesium sulphate Seawater with Specimens in Concentrated Seawater after 22 hours.

At least 9 crabs were used in each solution.

Solutions used and freezing points.		(°C).	△ of Blood (°C).
A	Concentrated seawater	3·06	2·18
	Seawater plus Mag. sulph.	3·05	2·185
B	Concentrated seawater	3·14	2·18
	Seawater plus Mag. sulph.	3·14	2·19
C	Concentrated seawater	3·14	2·29
	Seawater plus Mag. sulph.	3·15	2·3

TABLE 6.

Comparison of H. cordiformis in Concentrated Seawater with Specimens in Sugar and Urea Seawaters (duration of immersion 22 hours).

Solutions used and freezing points.		(°C).	△ of Blood (°C).
D	Concentrated seawater	3·38	Blood 2·45
	Seawater plus sugar	3·35	„ 2·50
E	Concentrated seawater	3·29	Blood 2·26
	Seawater plus urea	3·24	„ 2·46

Note. Attention is drawn to the fact that in each of the above experiments the parallel control in concentrated seawater was set up so that crabs from the same batch, collected at the same place and time, were tested together.

Since in all the above experiments the freezing point of the blood was depressed to approximately the same extent whilst the external media were of approximately the same osmotic pressure but very different in constitution, a comparatively simple osmotic effect—the withdrawal of water—would explain the results. But if such were the process one would expect the chlorine content of the blood to be the same in each case (2). Samples of blood from the experimental animals were analyzed with the following results:

TABLE 7.

	Chlorine in blood of crabs in normal seawater (grm. NaCl per litre).	Chlorine in blood of crabs in concentrated seawater (grm. NaCl per litre).	Chlorine in blood of crabs in seawater concentrated by addition of $MgSO_4$. (grm. NaCl per litre).
Expt. A.	24·9	28·4	25·1
Expt. F.	24·8	27·5	24·0
Expt. G.	25·0	28·4	24·5

These results are very definite. They show that the salinity of the blood in the two sets of experimental crabs was very different, notwithstanding that the freezing point was approximately the same in each case. They show clearly that the concentration of salts in the blood was not due to the withdrawal of water, because there was no concentration of NaCl in the blood except in those crabs living in the medium in which NaCl was concentrated. In the parallel examples in magnesium sulphate seawater the chlorine content of the blood remained about identical with that of normal crabs. All this fits in with the weight experiments. The lowered freezing point of the blood has then in each case been occasioned by the passage inwards respectively of NaCl or magnesium sulphate, or urea, or by the ions of NaCl and $MgSO_4$, to which the bounding membranes of *Heloecius cordiformis* are without doubt permeable.

This discovery provides confirmation by a somewhat different method, and experimenting with an entirely different species, of the analytical results of Bethe (1929).

The fact that in these experiments with external media of different composition, but like osmotic pressure, the blood samples presented the same freezing points, indicates that osmotic pressure has probably played some part. It is not likely, however, that the relations between the blood and external media are as simple as is frequently assumed. It will be noticed that isotonicity was not attained in a single case in the concentrated seawaters any more than in diluted seawater.

[2] It will be noticed that the normal chlorine content of the blood of *Heloecius cordiformis* in seawater of 34·0 salinity is considerably less than the amount in the surrounding medium, although the blood is isotonic. The chlorine analyses were made by the blood method of Van Slyke. (Journ. of Bio-Chem., Dec., 1923.)

Experiments with the Marine Pulmonate, Onchidium chamaeleon.

Aquatic invertebrates with soft body walls generally show a distinct change of weight when the salinity of their environmental media is changed. Their bounding membranes appear to have the properties of semi-permeable membranes. *Onchidium* is a marine pulmonate and as it happened to be present on the mangrove swamp in huge numbers it was utilized for experiment. Fortunately it was very amenable to considerable fluctuations in the salinity of its environment. This is the first occasion in which a member of this group of molluses has been thus exploited.

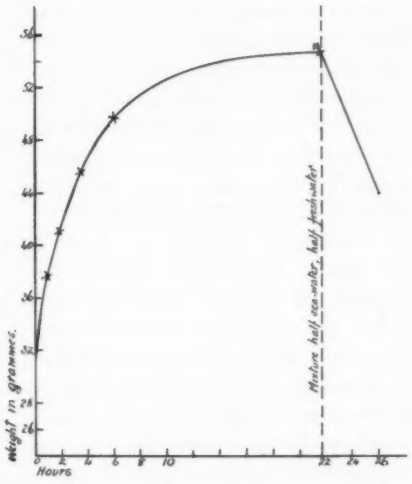

Figure 1. Weight of *Onchidium* in a diluted seawater (10 parts seawater, 90 parts fresh water). At X the specimens were transferred to a stronger mixture.

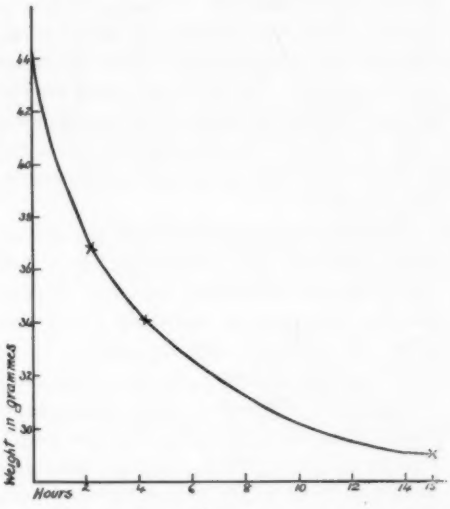

Figure 2. Change in weight of Onchidium in a solution isotonic with seawater ($\triangle 1{\cdot}95$), but made up of ⅓ seawater and ⅔ cane sugar solution.

The curve (figure 1) indicates that *Onchidium* behaves in diluted seawater as though the body-walls were semi-permeable membranes. When the Onchidia gained in weight in diluted solution they could easily be brought back to the original weight by replacing in normal seawater. Such experiments were repeated several times, and during the experiments the animals remained in good condition. The observations are directly comparable with those of Dekhuyzen (1920) on Sipunculids.

In 1930 Bethe brought forward what has been regarded as proof of the permeability of the body-wall of a "soft-skinned" invertebrate to salts or their ions.

The subject was the mollusc *Aplysia*. He placed specimens in a fluid which, whilst isotonic with normal seawater, consisted of a mixture, half being seawater and half a solution of cane sugar. Since this external medium was also isotonic with the blood, it was expected that no change in weight would occur in the specimens. Bethe found that the unexpected took place; a loss of weight ensued, and this continued until, as he puts it, "their blood became so viscous that death occurred." Bethe's explanation of the phenomenon is as follows: At the commencement the osmotic pressure is equal within and without the animal, but the concentration of sodium chloride is lower outside than in the blood. If we assume that the body-wall is actually permeable to sodium chloride or its ions a diffusion of the salt outwards would take place. This, however, would reduce the osmotic pressure of the internal medium below that of the external and consequently water would begin also to pass outwards, resulting in loss of weight. This process would continue, no equilibrium being possible. The assumption of permeability of the bounding membranes to salts is regarded as the only satisfactory explanation of the facts observed. The experiment of Bethe is so interesting that we sought confirmation on *Onchidium*, with the results shown in figure 2.

Onchidium responds to submersion in a solution of seawater and cane sugar made isotonic with the blood in a manner similar to *Aplysia* in Bethe's experiments. We have, however, carried the matter further by making up isotonic solutions of seawater containing magnesium sulphate and seawater plus urea solution. An experiment, in which the three different types of solutions were being used at the same time on three samples of Onchidia, is illustrated by figure 3. At least 1 dozen Onchidia were taken for each experiment. The results show how the Onchidia lost weight in the sugar-seawater and the magnesium sulphate water, the loss being less in the magnesium sulphate, but the weight of those in the urea seawater remained practically constant after a slight initial loss.

If we explain the loss of weight of the specimens in the sugar solution by assuming the outward diffusion of NaCl (or its ions), we must explain the loss of weight in the magnesium sulphate solution along the same lines, i.e., by postulating that the NaCl diffused outwards more rapidly than magnesium sulphate diffused inwards. This would also explain why the loss in weight of the specimens in magnesium sulphate seawater was not so great as that of the specimens in the sugar solution. Bethe himself shows that sugar diffuses inwards, though slowly. The rate of diffusion of magnesium sulphate might be expected to be less than NaCl but greater than cane sugar. The urea result may be interpreted by postulating a comparatively rapid penetration of the bounding membranes by urea. Our experiments on *Onchidium* may so far be accepted as confirming Bethe in regard to the permeability of bounding membranes to salts.

We must now return to Bethe's experiments on *Aplysia* and to some of those

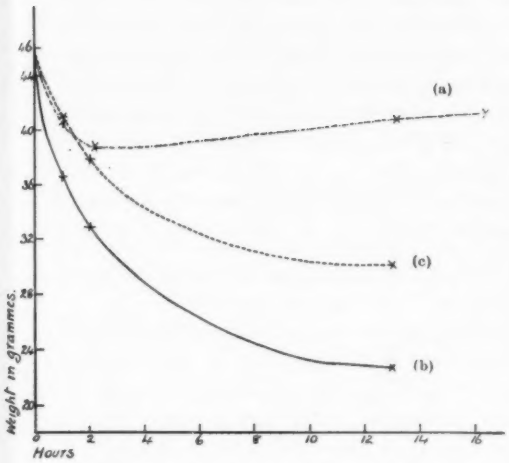

Figure 3. Change of weight of *Onchidium* in solutions isotonic with normal seawater (△ 1·95°C), but made up with
(a) ⅓ seawater, ⅔ urea solution.
(b) ⅓ seawater, ⅔ cane sugar solution.
(c) ⅓ seawater, ⅔ $MgSO_4$ solution.

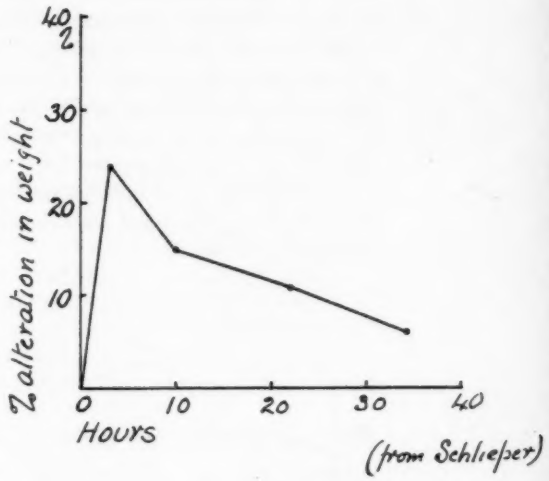

Figure 4. Percentage alteration in weight of *Nereis* in diluted seawater (from Schlieper, 1930).

of Schlieper with a species of *Nereis*. Bethe records that in certain experiments in which *Aplysia* taken from normal seawater was placed in diluted seawater the specimen first gained in weight (an expected result in the fluid of low osmotic pressure compared with the blood), but afterwards decreased again, returned to the original weight, and in some cases "the weight may even decrease still further." An example curiously like this is Schlieper's experiment with *Nereis* (his graph is illustrated in figure 4). Bethe states that this phenomenon (in *Aplysia*) is only explicable on the assumption that the first effect of the artificial change of environment is a diffusion inwards of water, but that this is followed by a transfer of salts outwards and a loss of water. It is difficult to see why the weight should remain *less* than it was originally.

We think this type of experiment needs considerably more supervision, especially in view of the result about to be described, and we are not prepared to accept these experiments of Bethe without further confirmation. Some light was thrown on this question by the behaviour of *Onchidium* in a urea seawater solution (see figure 5) which was isotonic with normal seawater, and thus with the creature's blood. Eleven specimens were used, and after an increase in weight from 74·8 to 81 grammes within the first 3 hours the weight remained very constant for 24 hours. During this period the Onchidia were very active, and in

apparently good condition, in the unusual medium. They all died, however, very suddenly somewhere between the 25th and 30th hour, and at the same time the weight dropped to 48·3 grammes. This extraordinary decrease was clearly due to great loss of water, and the specimens were contracted. Now we do not believe that this had anything to do directly with the natural permeability of the bounding membranes or with osmo-regulation. It is notoriously difficult to preserve "soft-skinned" invertebrates fully expanded. One needs only to think of the distorted specimens of nudibranchs, chaetopods, and anemones in our museums. Muscular contraction not only accompanies death, but is a sure indication of deleterious conditions.

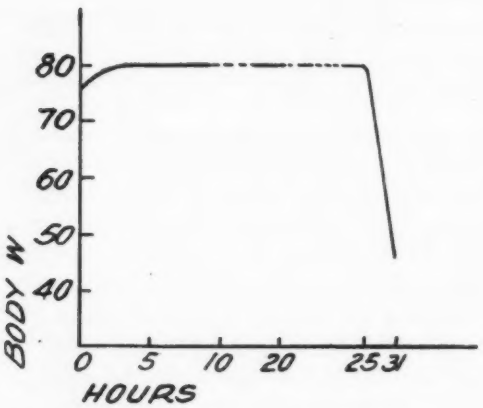

Figure 5. Change in weight of *Onchidium* in isotonic urea-seawater. Note sudden fall after 25 hours.

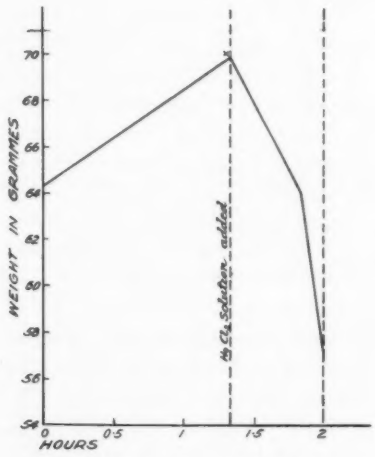

Figure 6. Change in weight of *Onchidium* in seawater of half normal salinity. $HgCl_2$ solution was added at X.

Acting therefore upon the suggestive nature of these observations, we placed a weighed number of healthy Onchidia in a mixture of equal parts of ordinary seawater and fresh water, and recorded their weight from time to time. It increased as was expected. (See figure 6.) Then at the end of 1½ hours (a safe period, since we had never seen *reduction* in weight take place in such a medium during 25 times this duration) a small quantity of concentrated corrosive sublimate was added drop by drop. The result was an immediate contraction, and during the following hour (after which the experiment was discontinued) the specimens continued to shrink and the weight to go down. We see no reason why any soft-skinned aquatic invertebrate should not lose in weight, notwithstanding

the osmotic concentration of the solution in which it is immersed, if that solution irritates and causes contraction, yet such a possibility does not seem to have been appreciated. We cannot say, of course, that this accounts for Schlieper's *Nereis* losing weight after its first increase, nor for Bethe's *Aplysia* becoming less heavy than it was at the beginning, although replaced in normal seawater. But knowing the sensitivity of the animals the result is suspicious.

SUMMARY AND DISCUSSION.

Before proceeding to discuss the substance of the experiments described above it is desirable to summarize the new facts obtained.

(1) It has been possible to find another marine invertebrate upon which extensive experiments have been made on a scale comparable with those on *Carcinus moenas* of Europe. The animal is a mangrove crab, *Heloecius cordiformis*, living not far from the sea, in water of high salinity, but subject to occasional freshenings of considerable extent after rains.

(2) The results of freezing point determinations of the blood, together with blood analyses for this and certain other crustacea, indicate that the condition of homoiosmoticity is more widespread than was supposed. In seawater which is diluted with fresh water the blood of *Heloecius cordiformis* is more saline than the surrounding medium, and the difference between the blood and the external medium becomes greater as the latter approaches fresh water. In other words, regulation of blood salinity is present.

(3) *Heloecius cordiformis* seems to show a greater independence than other marine invertebrates investigated up to the present, in that its blood tends to retain its normal saline constitution when the external seawater is made more concentrated. This is being further investigated.

(4) No changes in weight take place when the crab, *Heloecius cordiformis*, is placed in either dilute or concentrated seawater. The changes in the blood salts which accompany changes in the outer medium are likely then to be due to movements of salts or their ions inwards or outwards.

(5) This is confirmed by the fact that crabs in concentrated solution of equal osmotic pressure, but of different composition, i.e., highly concentrated seawater with normal proportions of salts, ordinary seawater plus magnesium sulphate, ordinary seawater plus sugar, ordinary seawater plus urea, have (after a time) approximately the same freezing points (and osmotic pressure) for the bloods, but the chemical composition is different in each case. Salts or ions have wandered inwards. The urea in the blood was quantitatively estimated[3] (in the urea seawater experiment), and found to be exactly that required to give the blood its new and lower freezing point.

[3] The urea analysis was made by converting the urea into ammonia with urease, and titrating the ammonia.

(6) The marine pulmonate, *Onchidium,* contrasts strongly with the hard-shelled crab, in that its weight increases in diluted seawater and decreases in concentrated seawater. Changes in the salinity of the external media are accompanied by changes in the body fluids, but the body-wall acts more like a semi-permeable membrane, and water movements through it are more facile than the passage of salts, which, however, also takes place.

(7) Experiments described above indicated that the results of some experiments in which soft-bodied marine invertebrates had *lost* weight in abnormal solutions of seawater should be regarded with caution. Muscular contraction resulting from *any* unfavourable conditions may cause expulsion of water and consequent loss in volume and weight.

It is evident that the more information is obtained about the relations between the internal fluids (body fluids and blood) and the environment, in the case of aquatic animals, the more complex is the regulation which seems to be involved.

The blood in the mammals is a fluid of remarkable constancy, and in the light of the present work we can see that the requirement of a certain constancy of composition in the fluids bathing the living tissues of the Metazoa is expressed more clearly than was hitherto suspected in the Invertebrata. The sea itself provides for marine invertebrates a medium whose constancy in saline composition is well known to oceanographers. It is no wonder that Bunge, Quinton, and above all Macallum, regarded the ocean as directly responsible for the particular saline composition of the blood of vertebrates, from the teleost fishes upwards. But the invertebrates also have their facilities for sustaining some constancy in the salinity of their internal media.

Regarded from the viewpoint of the higher vertebrate conditions, it is natural that the first workers on the saline composition and osmotic pressure of the blood of marine invertebrates considered these creatures as having no, or very little, independence of blood composition. In many cases the body fluids are little else than seawater with a small protein content and some amoeboid cells. And in the "highest" marine invertebrates, the Crustacea, the fact that the blood is (under normal conditions) practically isotonic with the surrounding seawater led to the conception that this isotonicity was simple and purely physical.

The teleost fishes, which contrasted so markedly with this, had clearly evolved some regulating mechanism, for whether in the sea or fresh water their blood salinity was totally unlike that of the surrounding medium, and varied in cases where the same species could move from one of these environments to the other within very narrow limits.

To this was added the puzzle of the invertebrates in fresh water. The fresh water molluscs have certainly a blood of extremely low salinity, but the crayfish sustains a blood salinity as high or higher than that of the freshwater teleosts.

Thus the crayfish came to be regarded as an aquatic invertebrate with some

sort of mechanism for regulating the blood salinity. Investigations on the marine crab, *Carcinus moenas*, showed that the blood of this crustacean was also somewhat independent of the external medium, but apparently only in waters of less concentration than seawater. The latest view expressed by Schlieper is that brackish and freshwater invertebrates in general are homoiosmotic, although only two animal species have hitherto been completely investigated. We agree with him in this. We have added three others to the list, *Heloecius cordiformis*, *Sesarma erythrodactyla*, and *Onchidium chamaeleon*. We have gained a considerable amount of information about this homoiosmoticity, but two questions remain unanswered. The first of these is whether it is now really practicable to classify aquatic invertebrates in regard to the osmotic relations of their blood (or other body fluids) with the external medium. In our opinion all grades of relationship exist. There is a conception (shadowing the early work in this branch) that the teleost fishes possessed definite organs for regulating the osmotic pressure of the blood which were not represented (either by analogous or homologous organs) in the invertebrates. But there is no indication anatomically that the freshwater crustacea possess blood-regulating organs which are not present in their marine relatives; and the osmotic conditions normally prevailing in these two groups are just as different as between a teleost and a purely marine crab.

We have examined three mangrove crabs from the same locality, and all show different facilities for living in diluted seawater. Another, a new and as yet undescribed crab, *Sesarma* sp. ?, which is an addition to the number of genera of freshwater crabs, and whose close relations of the same genus are brackish or marine, has completely adapted itself to fresh water; its blood exhibits the same independence as that of the crayfishes. Any regulation of blood salts must be regarded then as due to specific differences in the activity of tissues which are present in all these forms.

Again. marine invertebrates of all phyla show a wide range of susceptibility to the dilution of seawater with fresh, but exceedingly few have been accurately investigated in regard to their osmotic relationships. We do not regard it as logical to say that the fresh and brackish water species (which apparently stand changes in the salinity of the external medium) are provided with a means of blood regulation and to deny it to all those marine forms which, because they are affected pathologically by small changes in their environment, exhibit isotonicity as the general rule. This implies that there is little significance in Schlieper's statement that "Für die Stenohalinen Evertebraten des Meeres, d.h. diejenigen welche nur geringe Schwankungen im Salzgehalt des Aussenmediums ertragen können scheint dagegen das Gesetz der Poikilosmotizität annähernde Gültigkeit zu besitzen." The fact is that all the marine invertebrates, including those which go into brackish water, like *Carcinus moenas* and *Heloecius cordiformis*, are isotonic within a small range (and thus apparently poikilosmotic). Some of them can live in water of salinity outside this range, and others cannot. To say

that the former are able to endure because the blood salinity is regulated and they become homoiosmotic, whilst the latter die because such a mechanism is absent, is unwarranted at present. It is more likely that the restricted ones die because certain tissues cannot endure an altered *internal* medium, though the degree of alteration may be slight and not greater than the changes in the blood of brackish forms with wide range. Further confirmation of this is provided by the fact that *Leptograpsus variegatus*, usually found on the sea coast, dies in diluted seawater, although the salinity of its blood at death is less changed than that of specimens of *Heloecius cordiformis* put into the same dilution at the same time. So it is merely axiomatic to say that a marine invertebrate which is restricted to live in seawater of normal high concentration is poikilosmotic. It simply cannot live under the conditions where other species present the homoiosmotic state.

Another example may be referred to here, since we have conducted experiments on a pulmonate living in seawater. It would appear reasonable to assume that the land snails had evolved from aquatic forms, and we may take it that the freshwater pulmonates of to-day have secondarily returned to an aquatic environment. It is interesting then to note that whilst the truly freshwater pulmonates are able either to rid themselves of much excess water, or the osmotic inflow is low, the common land snail, *Helix pomatia*, when completely immersed in water, swells up and eventually dies in a water-logged condition. The well-known method of killing snails for dissection by immersing them completely in tap water in a full bottle is generally regarded as killing by asphyxiation, but there is reason to believe that the true explanation is that given above. The point is that in closely related genera which have adopted different environments there are very different responses to the same external medium.

One thing is quite clear, and that is the remarkable general need for the cells of a metazoan to be bathed by a saline medium with a certain balance of ions. The optimum salinity of this internal medium no doubt varies in different species, just as the actual salinity varies, and possibly the optimum is not always realized in freshwater organisms. This may be the limiting factor in migration into that medium. Those organisms which can retain the necessary saline body fluids, notwithstanding the semi-permeable body-walls (or parts of such) can live in fresh water, whilst those which are unable to retain salts in their body fluids, or whose optimum requirements for these is higher than the amount retainable, will be unable to invade fresh water.

In putting forward these views it seems natural to accept the idea that an aquatic animal whose internal fluids are *sheltered* from changes in the external medium (no matter what the means be, simple or complex) will be more successful in passing from one environment to another. In this connection we are reminded of the contrasting response of the two species, the hard-shelled crab and the slug with the soft body-wall discussed in this paper. It may be that insufficient attention has been paid to this feature. It is well worthy of note that a specimen

of *Heloecius cordiformis* will stand a change from seawater to fresh water without any change in weight, whilst under the same conditions soft-bodied animals increase greatly in weight, owing to the osmotic passage inward of water. This seems due, not to differences in the osmotic permeability of the body-walls, but to the fact that if a bounding membrane is inelastic and inextensible a passive resistance will be exerted against an inward flow of water which would bring about an increased volume within the body if not simultaneously secreted. An aquatic animal provided with such a body-wall would not expend so much energy in order to regulate the water content of its body. Thus it has been suggested that it is the pressure exerted by the somewhat rigid walled cells of plants—the resistance to a change in volume—which enables them to withstand a great latitude of osmotic pressure in their environment. It certainly seems as if our mangrove crab was protected to some extent in this way. Schlieper found that there was little change in weight of *Carcinus moenas* in diluted seawater, but he regards this as due (amongst other things) to an *active* resistance by the body-wall to an inward water flow, because four crabs which died during an experiment of this kind in dilute seawater increased in weight, and also because treatment of the gills with caustic soda caused a considerable increase in weight. We have not tried this latter kind of experiment yet, but none of our crabs which died during the course of the investigation ever increased appreciably in weight, so in this respect *Heloecius* must differ from *Carcinus*.

No investigators making observations on alterations in the volume and weight of aquatic animals in different fluids ever seem to have determined the actual quantity of water present in the animal at the beginning of the experiment. Without doing this it is not easy to estimate the significance of the weight changes. An average-sized specimen of the mangrove crab, *Heloecius cordiformis*, weighing 6·4 grammes, was killed without loss of blood and dried at 100°C. until the weight was constant. The loss in weight, representing the water of the tissues, was 4·2 grammes. This estimation provides additional confirmation that the changes in freezing point of the blood from the crabs in different dilutions (or concentrations) of seawater is due to the passage of salts or ions, for if it had been due to water-flow the weight of water required to bring about such changes in concentration should easily have been recognized.

We have diverged slightly from the question as to whether there is a complete absence in those marine invertebrates, restricted at present to seawater of normal concentration, of the means by which some independence of the blood salinity (and of osmotic pressure) can be sustained. It will be seen that we favour a view that it is impossible to draw a definite line in this regard between the freshwater, brackish water, and marine forms of any invertebrate class in which such a distribution is found, i.e., crustacea. Various types connect these oecological groups, whilst closely allied species may be quite unlike in their habitat and their reactions.

The second question is the more difficult one to answer. What are the factors concerned in sustaining a difference in osmotic pressure or in saline constitution between the external medium on the one hand and the blood and internal fluids on the other, when such are separated by a membrane which appears to be permeable to both water and salts? We are very far yet from a reliable conception of this phenomenon in any one case, and particularly so in regard to the invertebrates. The problem involves a knowledge of the phenomena of excretion (still a difficult subject in the case of the much-studied mammalia), together with all the mechanisms of diffusion through living and non-living membranes. The most we can do at present is to determine, if possible, the relative parts played by the different organs. This is not the place to enter upon a full discussion of the matter, because it involves reference to a great deal of work not touched upon in this paper. There are one or two points, however, upon which our work has a bearing. It must be clear now, for example, that when a marine invertebrate like *Carcinus* or *Heloecius* lives in much-diluted seawater (experimentally or naturally, and in so doing sets up a new blood equilibrium in which the osmotic pressure is substantially different from that of the external medium, the new conditions may set up *either* a tendency for an increased flow of water inwards through the body-wall, or of salts by diffusion outwards, or both. When the change is made suddenly there is a rapid alteration in the blood, and an appreciable loss of salts occurs within 48 hours. *It is during this period that it is possible to determine experimentally that the bounding membranes are permeable to salts.* But the attainment of a new equilibrium, in which the salinity of the blood remains constant (although much lower than when the creature was in seawater), indicates that the organism has balanced the new physico-chemical complex by control of the movements of water and salts. What exactly is now happening in the physiological functioning of the various organs that is different from the state of affairs when the creature was in seawater?

We may approach this question by a glance at certain conditions exhibited in the regulatory activity of the kidneys of the higher animal. Thus in man if water is taken in large quantities the water volume of the urine increases correspondingly, but there is little change in the blood. The amount of solids in the urine may decrease from 5% to 1% or less. Now the urine of a marine crab in its normal habitat is of approximately the same freezing point as the blood, whilst the excretory fluid of the freshwater crayfish, *Potamobius astacus*, is definitely much less saline than the blood. Recorded freezing points for crayfish blood average —0·8°C. to —1·0°C., and for urine —0·2°C. It would almost appear, therefore, as if the answer to the question were simple, and that the explanation of the independence of the salinity of the blood of freshwater crustacea was due to an increase in the output of water from the excretory organs.

This explanation will not do, however, or at least it is not of general application, for according to Schlieper the freezing point of the urine of *Carcinus* is

the same as that of the blood, whether the specimens are in normal seawater, with the blood △ 1·91°C., or in diluted seawater (—0·64°C. freezing point), with the blood△ 1·25°C. and urine △ 1·27°C. And this is not the only case of its kind, for Schlieper gives the △ of the urine of *Telphusa fluviatilis*, a freshwater crab, as averaging 1·16°C., when that of the blood is 1·16°C. There is a remarkable and totally unexpected discrepancy, therefore, between the composition of the urine in the freshwater crayfishes and the freshwater crabs. Unfortunately no analyses of the composition of the urine of Schlieper's crabs have been made, nor is any information available about the quantity of urine excreted in a given time. Until these figures are confirmed and amplified it is inadvisable to go further into the matter, except to say that it is impossible at present to see how *Carcinus* in diluted seawater can withstand the excretion of a moderately highly saline urine and still keep up an equilibrium in which the blood remains of relatively high salinity. We have kept *Heloecius cordiformis* for almost three months in diluted seawater (freezing point —0·72°C.), and without feeding the specimens. During this time the freezing point of the blood remained approximately constant. Unfortunately the specimens were too small to enable us safely to determine the urine concentration. One is forced to the conclusion that either there is a secretion of surplus water by the excretory organs (as in the crayfish; we have proved this to be the case for Australian crayfishes, which are of very different genera from the European species), or that when a new equilibrium is reached in the case of crabs there is very little passage of salts or their ions outwards through the body-wall.

The crayfishes present conditions not altogether unlike those of a frog submerged in fresh water. Under such circumstances there is a considerable increase in the secretion of water by the kidneys to balance the inward flow occurring through the skin. A frog is said to be able to excrete more than its body weight of water in 24 hours. But this skin inflow is not purely the physical process of osmotic flow through a non-living membrane. The skin exercises a resistance to the inward flow; it controls the amount of water which is allowed to enter (Adolf, 1930), the kidneys secrete the surplus. Similarly the body-wall of the crustacea may determine the movement through it of water and salts in such a manner that the process varies, not only with different species, but at different times and under different conditions.

Heloecius cordiformis resembles *Carcinus moenas* in diluted seawater, but is unusual amongst marine invertebrates in regard to its resistance to changes in the concentration of the external medium in the direction of higher concentrations. Even after a month in seawater of the abnormal salinity of 52‰ the △ of the blood had not reached that of the seawater. This interesting result reminds one of an experiment of Dekhuyzen with *Phascolosoma*. Dekhuyzen took the weight of this worm, after a short sojourn of 20 minutes in seawater diluted by 10%, and compared it with the weight after 20 minutes in seawater concentrated

by 10% through addition of NaCl. He found that there was a greater loss of weight in the concentrated water than gain in weight in the diluted water, and he described this as the exhibition of a defence by the epithelium against diluted solutions. The two sets of facts are not quite of the same order, but they indicate that any regulatory processes taking place when a marine invertebrate passes into diluted seawater are not reflected by corresponding processes on the concentrated side of the normal. A very definite regulation of the internal medium in a concentrated saline environment has been observed in one case, the brine shrimp, *Artemia*, where the salinity of the blood was permanently far below that of the external medium. We consider that other forms, including marine invertebrates, will be found to show varying independence in concentrated saline solutions.

From an oecological point of view it is worth emphasizing the extraordinary conditions that these marine crabs of the mangrove swamps will stand. We have placed them in jars of seawater of low concentration, and we have placed them in jars of water with concentrations so high that it is scarcely conceivable that such salinities would be reached in nature, for the tide washes regularly over the flats. Their normal existence includes a certain number of hours out of the water altogether. This has been impossible to arrange under the conditions of experiment, and they have not been fed during the experiments. Yet after $2\frac{1}{2}$ months these crabs were quite active in the diluted water, and equally active after 4 weeks in the concentrated seawater when that experiment was concluded.

Future work must determine the relative importance of the bounding membranes and the excretory organs in these regulations of blood salinity in aquatic animals. The work of other observers, as that of ourselves, stresses the great importance of the bounding membranes. It is interesting from this point of view to notice that the death of the teleost fish, *Gasterosteus aculeatus*, in pure sodium chloride solution (in contrast to the non-lethal character of such a solution when suitable quantities of calcium, potassium, and magnesium salts are added to it) has been shown recently (Krüger) to be due to the destruction of the bounding epithelium of the body. This is accompanied by a striking entrance of NaCl into the fish.

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THE ACTION OF DILUTE FORMALDEHYDE SOLUTION ON SOME AMINO-ACIDS

by

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In a previous paper (1) an account was given of some experiments on the action on certain proteins, proteoses, and peptones, of formaldehyde in low concentration. It was found that while in every case there was a decrease in the free amino-nitrogen as estimated by Van Slyke's method, in none was the disappearance complete. Considerable differences were observed in the percentage nitrogen remaining at the end of the reaction. It was therefore decided to submit some of the amino-acids to a similar experimental procedure to see whether any notable differences existed in their behaviour towards this reagent.

Amino-acids.

Representatives of the various groups of amino-acids were prepared in this laboratory. Glycine and alanine were synthetized by Strecker's method. Cystine was prepared from hair; glutaminic acid from wheat gliadin; lysine and histidine from blood corpuscles. The last was precipitated as its dinitro-naphthol-sulphonate according to Vickery (5). Tyrosine and a small amount of leucine were obtained from a tryptic digest of casein. The lysine sample contained some lysine carbonate, which formed during its preparation. Its amino-nitrogen content, estimated with a micro Van Slyke apparatus, was 17·5%, whereas that of the pure amino-acid is 19·2%. Both glycine and alanine contained small amounts of non-nitrogenous impurity. In the case of alanine it was sodium sulphate. The other amino-acids were apparently pure.

A difficulty was encountered in the amino-nitrogen estimation of some of the amino-acids by Van Slyke's micro-method. Six minutes was the time usually allowed for the reaction, shaking the reaction-chamber throughout. When the laboratory temperature was high five minutes' shaking was sufficient. For the estimation of lysine the apparatus was shaken at first to ensure complete mixing, and for the last six minutes of the reaction period of a half-hour. Histidine, alanine, leucine, and lysine gave a constant value for amino-nitrogen

content. Glycine, cystine, and tyrosine evolved larger volumes of gas than theory requires. This fact is noted for glycine and cystine by Plimmer (3), who states that the true value for glycine may be obtained by subtracting 3% from the total volume of gas, and for cystine by multiplying by the factor 0·926. These variations were supposed to be constant. Higher values, however, were obtained for the amino-nitrogen of both these acids. When the laboratory temperature was higher than 25°C., sufficient gas was evolved during a cystine estimation to give a value of 13·6% amino-nitrogen, as against the theoretical value of 11·66%, an excess of 16·6%. Glutaminic acid at a temperature of 27°C. gave an amino-nitrogen percentage of 9·83% instead of 9·52%. At lower temperatures this estimation is satisfactory. Van Slyke (4) records an estimation of 7·89% amino-nitrogen for tyrosine. This amino-acid was found to give values from 7·7% to 9·5%, depending on the time of reaction and the temperature of the laboratory (Table 1). The tyrosine had been recrystallized twice, and well washed with distilled water. It did not react with Nessler's reagent nor with boiling caustic soda and lead acetate, indicating freedom from ammonia and cystine. Its total nitrogen, estimated by Kjeldahl's method, agreed with its formula. Table 1 indicates that, like cystine and glycine, tyrosine does not react with nitrous acid on the lines of the simple equation assigned to it.

TABLE 1.

Tyrosine. Estimated by Van Slyke's Method.

Theoretical Percentage of Amino-nitrogen 7·73.

Weight. of tyrosine.	Solvent.	Time of shaking.	Cc. Nitrogen. evolved.	Temperature.	Pressure in mm. Hg.	Percentage amino-nitrogen.
9·643	dil. HCl	7 mins.	1·269	12°C.	763	7·80
6·140	" "	6 "	0·860	18°C.	770	8·18
6·261	" "	8 "	0·902	18°C.	770	8·38
5·909	dil. NaOH	4 "	0·790	16°C.	771	7·87
7·441	" "	6 "	1·050	19°C.	770	8·19
7·102	" "	8 "	1·041	19°C.	770	8·49
6·567	" "	7 "	0·930	22·5°C.	764·5	8·02
9·088	" "	5 "	1·330	20°C.	754	8·26
7·148	" "	6 "	1·122	28°C.	762	8·58
8·250	" "	after ½ hour, 10 mins. shaking	1·377	20°C.	754	9·42

Total nitrogen estimations (micro-Kjeldahl) were made of those amino-acids, which behaved irregularly with respect to the Van Slyke amino-nitrogen determinations. They gave theoretical values except in the case of glycine and alanine. The latter gave the same value for total as for amino-nitrogen.

TABLE 2.

Total Nitrogen Estimations.

Amino-acid.	Percentage total nitrogen.
Cystine ..	11·65
Tyrosine ..	7·74
Glutaminic acid ..	9·56
Glycine ..	18·13
Alanine ..	15·00

The Reaction with Formaldehyde.

The amino-acids were heated to 37°C. in 1% solution with 0·5% formaldehyde. Toluene was used as a preservative, and control solutions were similarly heated without the addition of formaldehyde. Cystine, tyrosine, and leucine dissolved in dilute hydrochloric acid and glycine dissolved in water, were first tested. No decrease in the amino-nitrogen percentage was observed over a period of two months. Then it was found that if these amino-acids were dissolved in dilute sodium hydroxide solution a progressive decrease in amino-nitrogen followed the treatment with formaldehyde. Alanine and glutaminic acid behaved similarly. Formaldehyde reacted with the amino-nitrogen only in alkaline solution. Lysine and histidine reacted in aqueous solution. The lysine solution was at pH 9·6, the histidine at pH 7·4. In reacting with formaldehyde in practically neutral solution histidine contrasts sharply with the other amino-acids studied.

A series of experiments was then made, heating with 0·5% formaldehyde 1% solutions of the amino-acids dissolved in 0·1 N. sodium hydroxide. This was the minimal concentration of alkali which would readily dissolve cystine and tyrosine in 1% solution. Lysine and histidine were dissolved in water. The tubes containing the solutions were sealed with paraffined corks, and estimations of amino-nitrogen of the controls and the formolized solutions were made periodically. Amino-nitrogen estimations were made of the control amino-acid solutions at the same time as of the formolized ones. This was necessitated by the varying figures obtained for the control amino-nitrogen percentage of tyrosine, cystine, and glycine. The amino-nitrogen of the formolized solutions was expressed as a percentage of the figure obtained for the control solution on the same day. Again, the amino-acids, especially cystine, are somewhat unstable in alkaline solution. There is a gradual decrease in the amino-nitrogen percentage. The amount of leucine obtained was insufficient for a complete investigation. A single experiment showed that in 14 days 57% of the amino-nitrogen was combined with formaldehyde; in 28 days 76·4%. The results for the other amino-acids are tabulated in Table 3, and plotted graphically to show the relative times taken by the amino-acids to combine with formaldehyde. (Figure 1.)

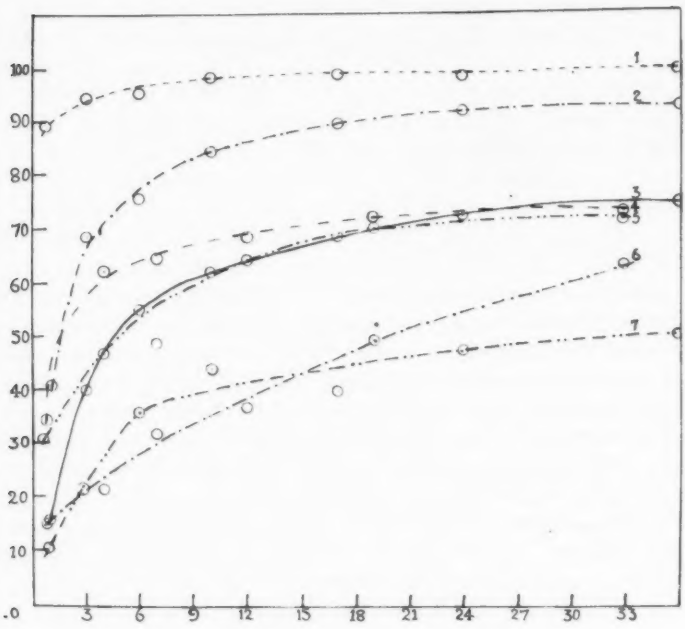

Figure 1.

Ordinates=% combination with formaldehyde.
Abscissae=No. of days heated to 37°C. with 0·5% formaldehyde.

Graph No. 1=Histidine.
2=Tyrosine.
3=Alanine.
4=Lysine.
5=Cystine.
6=Clycine.
7=Glutaminic Acid.

If the amino-acids are treated with formaldehyde in a higher concentration of H ions the percentage combination of free amino-nitrogen is less. Some experiments were made with glycine and lysine. At pH 7·0 only 22·4% of the amino-nitrogen of lysine had combined with formaldehyde after 12 days. In 19 days it had increased to 24·9%. It is interesting that there is any reaction at this pH, as glycine and alanine gave a negative result when so treated. At pH 8·2 15·3% of the amino-nitrogen of glycine reacted with formaldehyde in 7 days, and 18·3% in 14 days.

TABLE 3.

Results for Amino-acids treated with 0·5% Formaldehyde.

Tyrosine.				Alanine.				Histidine.				Glutaminic Acid.			
	Amino-nitrogen percentage.				Amino-nitrogen percentage.				Amino-nitrogen percentage.				Amino-nitrogen percentage.		
Time of reaction.	Control.	After heating with CH_2O	Combined with CH_2O	Time of reaction.	Control.	After heating with CH_2O	Combined with CH_2O	Time of reaction.	Control.	After heating with CH_2O	Combined with CH_2O	Time of reaction.	Control.	After heating with CH_2O	Combined with CH_2O
22 hours	8·69 8·58	5·16 5·09	40·6	24 hours	14·90 14·95	12·69 12·52	15·6	18 hours	9·08 8·98	1.00 0·95	89·2	21 hours	9·87 9·79	8·82 8·76	10·6
3 days	7·77 7·77	2·42 2·40	68·7	3 days	14·90 14·80	8·98 8·90	39·8	3 days	9·01 8·95	0·49 0·49	94·5	3 days	9·52 9·38	7·47 7·38	21·4
6 days	7·82 7·80	1·89 1·92	75·7	6 days	14·91 14·95	6·74 6·74	54·9	6 days	9·01 8·99	0·39 0·42	95·6	6 days	9·53 9·43	6·01 6·17	35·8
10 days	8·04 8·02	1·20 1·27	84·6	10 days	14·93 14·74	5·62 5·63	62·2	10 days	8·97	0·13	98·6	10 days	8·88 8·90	5·08 4·93	43·8
17 days	8·18 8·12	0·79 0·85	89·9	17 days	14·84 14·88	4·46 4·50	69·9	17 days	8·93 8·97	0·04 0·08	99·3	17 days	8·78 8·71	5·29 5·28	39·6
24 days	7·84 7·68	0·69 0·50	92·5	24 days	14·65 14·45	3·99 3·94	72·7	24 days	8·81	0·09	99·0	24 days	9·08 9·10	4·77 4·70	47·9
36 days	7·45 7·37	0·52 0·49	93·2	36 days	14·56 14·53	3·61 3·55	75·4	36 days	8·80	0·00	100·0	36 days	9·05 9·10	4·46 4·38	51·3

Cystine.				Glycine.				Lysine.			
15 hours	13·57 13·53	9·32 9·45	30·8	17 hours	18·97 18·94	16·19 16·21	14·8	19 hours	17·64 17·43	11·65 11·40	34·6
4 days	13·28 13·05	6·96 7·10	46·6	4 days	19·50 19·25	15·33 15·14	21·3	4 days	17·58 17·35	6·62 6·57	62·3
7 days	11·97 11·80	6·19 6·00	48·8	7 days	18·94 18·81	12·84 12·89	31·8	7 days	17·20 17·10	6·18 5·86	64·9
12 days	11·90 11·99	4·16 4·36	64·3	12 days	18·83 18·90	11·94 11·94	36·7	12 days	17·14 17·24	5·30 5·40	68·9
19 days	11·93 11·91	3·44 3·65	70·3	19 days	19·03 18·83	9·62 9·44	49·7	19 days	16·87 16·90	4·65 4·72	72·3
33 days	10·10 10·23	3·03 2·59	72·3	33 days	17·19 17·35	6·28 6·12	64·0	33 days	16·48 16·25	4·32 4·23	73·9

Histidine is the only amino-acid examined which loses all its amino-nitrogen during the course of the experiment. After 36 days a Van Slyke estimation shows no amino-nitrogen. The formolized product is less soluble than histidine, and slowly crystallizes out. As 89% of the amino-nitrogen combines with formaldehyde overnight, an experiment was made with histidine, estimating the amino-nitrogen percentage at much smaller time intervals. At the commencement of the reaction the rate of combination with formaldehyde is very rapid indeed. The solution of histidine was warmed to 37°C. and the formaldehyde added. Immediately, a sample sufficient for two estimations was withdrawn and placed in a tube in an ice-bath. In spite of keeping the solution between the duplicate estimations at this low temperature the amino-nitrogen value of the second duplicate was considerably lower than that of the first.

A greater percentage of the amino-nitrogen of proteose and peptone solutions combines with formaldehyde when they are heated in alkaline solution

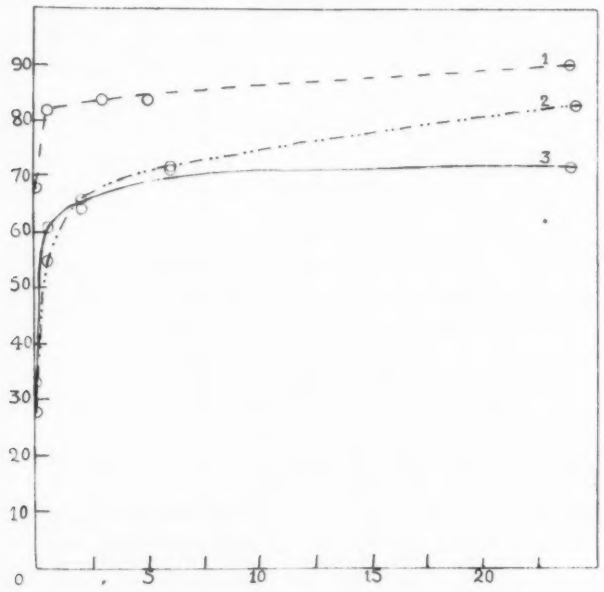

Figure 2.

Ordinates=% combination with formaldehyde.
Abscissae=No. of hours heated to 37°C. with 0·5% formaldehyde.
Graph No. 1=Histidine.
2=Primary Proteose.
3=Peptone.

than when heated in solution in water. Dissolved in water approximately 50% of the amino-nitrogen of peptone so combines (1). Experiments were made of the rate of combination with formaldehyde of the amino-nitrogen of a proteose and of a peptone solution dissolved in sodium hydroxide at pH 9·5. The concentration of the peptone solution was 1%, and that of the proteose 2%. The graphs resemble that of histidine. (Figure 2.) Results are given in Table 4. After 24 hours the rate of any further reaction is very slow. This is also shown in a prolonged experiment with the primary proteose "Berna" commercial peptone dissolved in water, and heated with 0·5% formaldehyde. After 7 months the proteose still had 0·15% of amino-nitrogen, compared with 0·86% amino-nitrogen of the original proteose.

TABLE 4.

Results for Histidine, Peptone, and Proteose Solutions treated with 0·5% Formaldehyde.

Histidine.				Peptone.				Proteose.			
	Amino-nitrogen percentage.				Amino-nitrogen percentage.				Amino-nitrogen percentage.		
Time of reaction.	Control.	After heating with CH_2O	Combined with CH_2O	Time of reaction.	Control.	After heating with CH_2O	Combined with CH_2O	Time of reaction.	Control.	After heating with CH_2O	Combined with CH_2O
On immediate addition of formaldehyde.	9·08 9·10	2·94 [2·21] After 20 mins. in ice.	67·7	On immediate addition of formaldehyde.	2·88 2·82	2·07 [1·79] After 20 mins. in ice.	27·4	On immediate addition of formaldehyde.	1·23 1·24	0·82 0·83	33·2
½ hour		1·64 1·66	81·8	½ hour		1·12 1·13	60·9	½ hour		0·55 0·57	54·7
3 hours		1·51 1·51	83·4	2 hours		1·05 1·02	64·0	2 hours		0·43 0·42	65·6
5 hours		1·49 1·51	83·5	6 hours		0·85 0·82	71·0	6 hours	1·24	0·37 0·34	71·3
24 hours	9·05 9·09	0·98 0·97	89·3	24 hours	2·76	0·82 0·77	71·2	24 hours	1·19 1·11	0·21 0·20	82·2

Some solid formolized tyrosine was prepared by heating an alkaline solution to 37°C. for one month. At pH 5·5 the bulk of the substance is precipitated. A further quantity of solid can be obtained by precipitation with mercuric sulphate, removal of the mercury with sulphuretted hydrogen, concentration on a water bath, and drying in a vacuum desiccator. The substances obtained by both methods are only slightly soluble in water, but freely soluble in dilute sodium hydroxide to a bright yellow solution; they are precipitated by acid from this solution at pH 5·5 and dissolve again at about pH 2. They contain no free formaldehyde, and give no blue colour on testing for free amino-nitrogen with

the ninhydrin reagent. Van Slyke estimations of their amino-nitrogen content give very low figures.

Optical Rotations of Formolized Amino-acids.

The specific rotations of the amino-acids were too small to permit accurate reading in 1% solution, with the exception of cystine and histidine. Polarimetric readings were made on solutions of these substances in 1 dm. tubes, using light of λ 5461 Å.U. Histidine showed no change in its specific rotation of —41° after heating with formaldehyde for 9 days, during which time the amino-nitrogen percentage decreased from 9·02% to 0·12%.

In the case of cystine there was a small decrease in rotation following heating with formaldehyde. After 10 days heating a control solution had a specific rotation of —121°, compared with —117° of the formolized solution containing 2·7% of free amino-nitrogen. After 15 days further heating the specific rotation of the control solution was —120°, and of the formolized solution —95°. Another sample showed a smaller decrease. After treatment with formaldehyde for 2 months the specific rotation was —107°, the control rotation still being —119·5°. The constancy of the control value is surprising, as cystine is not stable in alkaline solution, the Van Slyke amino-nitrogen percentage having fallen in the last case from 13·5% to 7·8%, without any change in the specific rotation.

Reversibility of Formaldehyde-Protein Reaction.

Some attempts were made to discover if the combination of formaldehyde with proteins were reversible. A formolized protein of known amino-nitrogen content was heated to 37°C. with a solution of an amino-acid which would combine readily with formaldehyde. After removal of the amino-acid the amino-nitrogen of the protein was again estimated. To facilitate the removal of the amino-acid the protein had to be readily precipitated while the amino-acid remained in solution. The metaprotein separated from "Berna" commercial peptone was used as the protein. It was reprecipitated once and dried in a vacuum desiccator. Lysine and histidine were the amino-acids chosen, as they are soluble in water, and combine readily with formaldehyde. Ammonia in the form of pure recrystallized ammonium sulphate was also tried.

The metaprotein was dissolved in 2% solution in 0·1% NaOH. Half was kept as control, the remainder being treated with 0·5% formaldehyde. After heating at 37°C. for 5 to 7 days the metaprotein in both solutions was brought to its iso-electric point with dilute acetic acid, centrifuged down, and washed with water and with alcohol and dried in a vacuum desiccator. In this way all traces of free formaldehyde were removed from the solid formolized metaprotein. The dry solids were again dissolved to a concentration of 2% in 0·1% sodium hydroxide. Heating for about half an hour on a boiling water bath

was necessary to effect the solution of the formolized metaprotein. The control was similarly treated. Amino-nitrogen estimations were carried out on both solutions with the micro Van Slyke apparatus. Then sufficient solid histidine or lysine was added to a portion of both solutions to make them 1% with respect to the amino-acid. Ammonium sulphate was added to make a 2% solution. Control solutions of the original and formolized metaprotein were kept without the addition of any amino-acid or ammonium sulphate. These solutions were then heated at 37°C. for 1 to 2 weeks. The metaprotein was separated out, washed, and dried as before. Solutions of the various portions were made in 0·1% sodium hydroxide by heating on a water bath. Van Slyke amino-nitrogen estimations were again made. In every case there was an increase in the amino-nitrogen content of the formolized metaprotein on treatment with the amino-acid or ammonium sulphate, without a corresponding increase in the amino-nitrogen of the control solution. In two cases the amino-nitrogen of the control solution decreased during the experiment. This may be due to removal of proteose from the metaprotein by its repeated reprecipitations. It is most difficult to separate completely the metaprotein from proteose. Results are given in Table 5.

TABLE 5.

Reversibility of Protein-Formaldehyde Reaction.

	Control Solution of Metaprotein. No Formaldehyde. Amino-Nitrogen Percentage.			Solution of Metaprotein. Treated with Formaldehyde. Amino-Nitrogen Percentage.			
Initial.	After heating alone.	After heating with $(NH_4)_2SO_4$.	After heating with amino-acid.	Initial after formal-dehyde treatment.	After heating alone.	After heating with $(NH_4)_2SO_4$.	After heating with amino-acid.
(1)							
1·10	—	0·74	0·80	0·37	—	0·47	0·69
1·16	—	0·74	0·84	0·38	—	0·49	0·70
(2)							
0·72	0·72	0·69	0·70	0·23	0·22	0·34	0·33
0·71	0·74	0·70	0·69	0·24	0·25	0·31	0·34
(3)							
0·69	0·52	—	0·54	0·16	0·18	—	0·36
0·67	0·53	—	0·53	0·17	0·19	—	0·37

Sample 1 was heated with 1% lysine for 10 days.
Sample 2 was heated with 1% histidine for 7 days.
Sample 3 was heated with 1% histidine for 14 days.

DISCUSSION.

The observations recorded in this paper would appear to suggest that the velocity of reaction of an amino-acid with formaldehyde is much influenced by its constitution. Thus histidine and tyrosine react more rapidly than the

simpler amino-acids. The case of histidine is remarkable in that it possesses a greater affinity for formaldehyde than proteoses and peptones, which themselves react far more readily than the simple amino-acids. The lack of any reaction in acid solution may perhaps be taken to indicate that the reaction occurs between formaldehyde and the undissociated amino-group. In this connection reference may be made to the work of Hewitt (2). He finds that at a pH, which he does not state, peptone and glycine react with formaldehyde only until some 15% of the amino-nitrogen has disappeared. The difference between his results and those described above may well be entirely due to the effect of different concentration of hydrogen ions employed.

SUMMARY.

The reaction between formaldehyde and amino-acids has been studied.

The influence of hydrogen ion concentration on the reaction is noted.

Little change in optical rotation during the action was observed.

The reaction of formaldehyde with metaprotein is reversible to some extent.

The effect of high laboratory temperatures on the estimation of amino-nitrogen with Van Slyke's apparatus is noted.

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A SIMPLE MICRO-BALY TUBE FOR ABSORPTION SPECTRUM ANALYSIS

by

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In the course of the investigations into the protein complex of the haemoglobin molecule, Hicks and Holden (2) were confronted by a difficulty due to the absorptive power of solutions of haemoglobin and sodium hydrosulphite. Since, even with a 5 mm. absorption tube, measurement in the ultra-violet proved impossible, some form of micro cell seemed desirable.

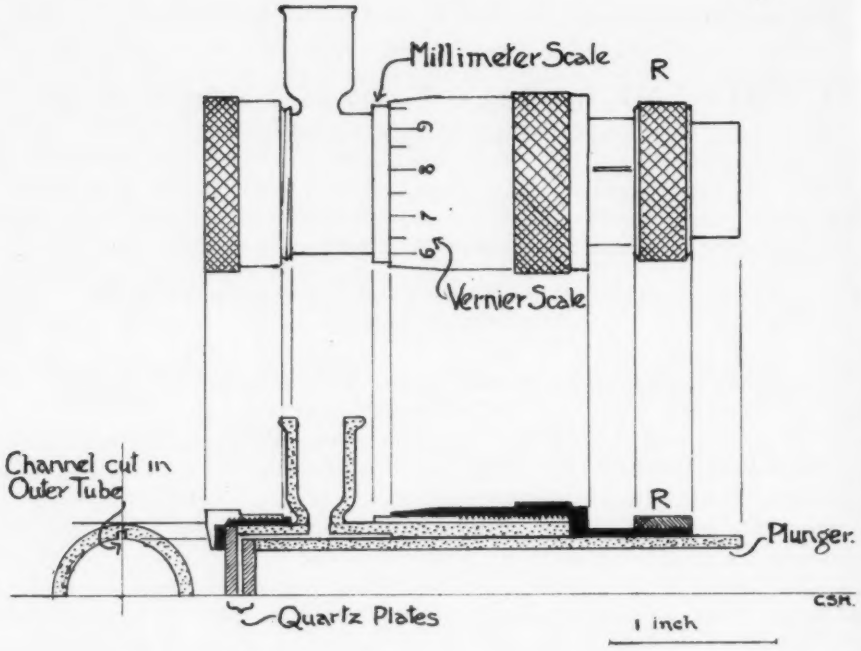
The design for the apparatus was based upon that of the Baly (1) adjustable cell. It was submitted in March, 1930, to F. Hellige & Co., of Freiburg, in Breisgau, who constructed the same to specification for the small sum of R.M. 50 each.

The cell is made so as to be available for investigation of both ultra-violet and infra-red absorption. For the former, the quartz end to the sliding member is attached by acid and alkali fast cement. For infra-red work, the spare tube is fitted with rock salt or sylvine plates, as required. The design has the advantage of combining reasonable accuracy of measurement of the thickness of the layer of liquid with ease of manipulation for cleaning, etc.

The divided drum is $1\frac{3}{32}$ inch in diameter, and is divided into 20 divisions; one rotation of the drum separates moving and fixed cell walls by 1 mm. Readings can thus be made to 0·05 mm. with a degree of accuracy depending upon the excellence of the screw thread.

To set the instrument, the movable quartz-ended member is pushed in until Newton's rings are to be seen at the front quartz plate with the divided ring set at zero. The clamp screw ring R is then tightened, and the cell filled by means of the cup, the desired thickness of layer being determined by a second setting of the graduated drum. This cell has an aperture of 15 mm. and a range of thickness variation from 0 to 10 mm.

The plungers are so well ground that there is no escape of fluid even at maximum extension.



The cells are used in connexion with Hilger's Judd Lewis sector photometer, and have resulted in a reduction of exposure time as well as the production of better photographs in the region of shorter wave lengths.

Since using this apparatus the firm of Adam Hilger Ltd. (3) has catalogued micrometer adjustment cells of a different design. The advantages claimed for the present type is accuracy of the zero setting, which is independent of the micrometer, the latter being used merely to read differences; at the same time the design is more compact and less costly.

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